*Xho*I/*Sal*I (filled in with Klenow) linearized pRS416 CYC1 (Mumberg, *et al.*, *Gene* **156**:119-122 (1995)).

MB1644 and MB2478 are *URA3*-based *S. cerevisiae* expression plasmids that contain the wild-type *lovE* gene. They are both derivatives of MB1199. MB1199 was created by using primers MO841 (SEQ ID NO:11) and MO842 (SEQ ID NO:12) to amplify the *lovE* ORF from *A. terreus* cDNA. Gateway (Invitrogen™, Carlsbad, CA) Cloning Technology (US Patent 5,888,732) was used to clone the *lovE* PCR fragment into the gateway entry vector pDONR206 (Invitrogen™, Carlsbad, CA) to create MB1199. Similarly, Gateway Cloning Technology was used to transfer the *lovE* ORF from MB1199 into MB968 to create MB2478 and into MB969 (U.S. Serial No. 60/198,335, filed April 18, 2000) to create MB1644.

MB2848 is a derivative of MB968 that contains a *lovE-AT274* chimera. The *lovE* portion of MB2848 was derived by using oligos MO841 (SEQ ID NO:11) and MO2278 (SEQ ID NO:13) to PCR amplify the *lovE* DNA binding domain from *A. terreus* cDNA. A second round of PCR was performed with primers MO343 (SEQ ID NO:14) and MO2278 to add appropriate Gateway Cloning Technology compatible sequences. The *At274* portion of MB2848 can be derived by using primers MO2273 (SEQ ID NO:15) and MO2274 (SEQ ID NO:16) to PCR amplify the carboxy-terminal domain of *At274* from *A. terreus* cDNA. A second round of PCR was performed with primers MO344 (SEQ ID NO:17) and MO2273 to add appropriate Gateway Cloning Technology compatible sequences. The *lovE* and *At274* PCR products were cut with *Bam*HI and purified over a QIAquick PCR purification kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Finally, the products were mixed 3-4 hours in a standard ligation reaction and used in Gateway entry and destination reactions to create MB2848.

Gateway cloning technology was used to clone the lovE variants of interest into plasmid MB1419 which is a filamentous fungal expression vector. The MB1419 fungal selection marker is the *A. nidulans GPD* promoter controlling the *ble* gene from *S. hindustanus*. The

transgene is controlled by the *A. nidulans PGK* promoter. *A. terreus* strain MF117 is a derivative of *A. terreus strain* ATCC 20542.

Example 2: PCR Mutagenesis of the *lovE* DNA Binding Domain

The zinc finger DNA binding domain of *lovE* is encoded by nucleotides 100-201 (SEQ ID NO:92). Oligos MO2624 (SEQ ID NO:18) and MO2654 (SEQ ID NO:19) were used to PCR amplify a *lovE* containing fragment from plasmid MB2478. The 1.7 kb product contains nucleotides 212-1410 of *lovE* and ~500 bp of flanking vector sequence. Two rounds of standard PCR (1.5 mM $MgCl_2$) were performed with Amplitaq DNA polymerase (Applied Biosystems, Foster City, Ca) according to the manufacturer's instructions.

Plasmid MB2848 was cut with *Kpn*I-*Bam*HI to release a 1.1 kb fragment containing the *At274* portion of the *lovE-At274* chimeric open reading frame. The remaining 5.5 kb vector sequence retains the *lovE* DNA binding domain.

Example 3: PCR Mutagenesis of the *lovE* Open Reading Frame

lovE open reading frame insert was prepared according to the following procedure. Oligo pairs MO2680 (SEQ ID NO:20) /MO2686 (SEQ ID NO:21), MO2681 (SEQ ID NO:22) /MO2686, and MO2700 (SEQ ID NO:23) /MO2701 (SEQ ID NO:24) were used to PCR amplify the entire *lovE* open reading frame from plasmid MB2478. The PCR products differ in the amount of 5' and 3' vector sequence flanking the *lovE* open reading frame.

PCR was performed using a GeneMorph PCR mutagenesis kit (Stratagene, La Jolla, Ca) according to manufacturer's instructions to achieve medium and high range mutation frequencies.

Plasmid MB2478 was cut with *Asp*718/*Xba*I to release a 1.7 kb fragment. The remaining 5.0 kb vector sequence completely lacks *lovE* ORF sequence.

Example 4: Transformation and Selection for G418R Isolates

All PCR products were purified using a QIAquick PCR purification kit (Qiagen) according to manufacturer's instructions. All vectors were gel purified using a QIAquick gel extraction kit (Qiagen) according to manufacturer's instructions.

The mutagenesis strategy of Muhlrad *et al.* (*Yeast* **8**:79-82 (1992))was used which involves cotransforming a mutated PCR product and gapped plasmids into *S. cerevisiae*, and then screening for *in vivo* recombinants having the desired phenotype).

Transformation of *Saccharomyces cerevisiae* was accomplished by the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/ss-DNA/PEG) protocol (Woods R.A. and Gietz R.D. *Methods Mol. Biol.* **177**:85-97 (2001)) with a 1:5 molar ratio of vector:insert DNA to generate >55,000 *in vivo* recombinant transformants on SC-Ura plates. Transformants were transferred by replica printing to YPD plates containing 100 µg/ml G418 and allowed to grow for 2-4 days at 30°C (Figure 1).

Drug resistant clones were confirmed in secondary assays including growth on G418 concentrations up to 2000 µg/ml. The plasmid-dependence of the phenotype was determined by observing the re-appearance of drug sensitivity correlating with loss of the library plasmid. *lovE* variant plasmids were recovered from promising candidates (Hoffman and Winston (1986) *Gene* 57:267). More than 70 *lovE* variants were identified and definitively characterized by DNA sequence and/or restriction digestion analysis.

Table 3 summarizes the G418 resistance phenotype and sequence analysis of 26 of these variants.